

A NOVEL SYNTHESIS OF FUMARIC ACID-1-¹⁴C

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SUMMARY

A simple, two-step synthesis of fumaric acid-1-¹⁴C from radioactive potassium cyanide and methyl 2-chloroacrylate is described. The intermediate products, methyl cis and trans 3-cyanoacrylates and the cis and trans 3-cyanoacrylic acids, were hydrolyzed directly to fumaric acid-1-¹⁴C in 55-60% yields. D,L-malic acid-COOH-¹⁴C was also obtained as a byproduct in 9% yield. A reaction mechanism is proposed.

INTRODUCTION

Labeled fumaric acid is a versatile precursor for several compounds of chemical and biochemical importance, e.g. maleic acid, maleic anhydride, N-ethylmaleimide, etc. Several procedures have been published for the preparation of fumaric acid-1-¹⁴C starting either with labeled potassium cyanide or with labeled carbon dioxide.

Nystrom *et al.*¹ utilized the four-step sequence, 1,2-dibromoethane \longrightarrow succinonitrile-1-¹⁴C \longrightarrow succinic acid-1-¹⁴C * \longrightarrow 2,3-dibromosuccinic acid-1-¹⁴C \longrightarrow fumaric acid-1-¹⁴C² which formed the desired compound in 63-76% yield based on potassium cyanide. Although excellent yields are realized, this method is lengthy and time-consuming. The use of sealed tubes in the bromination step (96 hour reaction time at 100°) is hazardous and presents limitations to scale-up. Benson *et al.*³ and Jorgensen *et al.*⁴ proceeded from labeled acetic acid to diethyl oxalacetate. Catalytic hydrogenation of the diethyl ester followed by hydrolysis produced a mixture of succinic acid-1-¹⁴C (28% yield) and D,L-malic acid-4-¹⁴C (39% yield). The latter was thermally dehydrated to fumaric acid-1-¹⁴C in 81% yield⁴. Fumaric acid, both 1-¹⁴C and 2-¹⁴C, has also been prepared from the appropriately labeled ethyl bromacetate via condensation with sodium diethyl malonate. The resultant triester was brominated and then subjected to acid hydrolysis to effect concomitant hydrolysis and dehydrohalogenation to fumaric acid in 8% yield⁵.

DESCRIPTION OF THE PROCEDURE

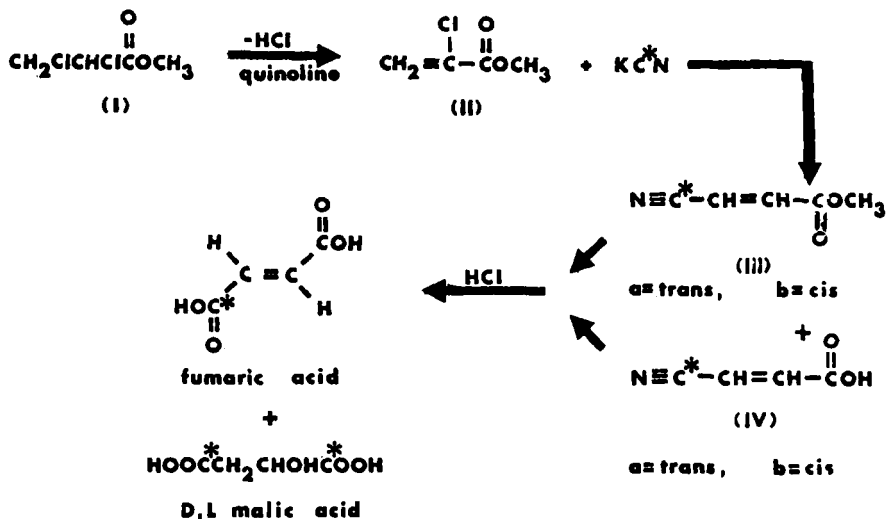
The present procedure represents a brief, two-step synthesis of fumaric acid, unequivocally labeled in the C-1 position, starting with methyl 2-chloroacrylate as shown in Scheme 1:

The starting material (II), is commercially available, but undergoes relatively facile polymerization. Freshly distilled methyl 2-chloroacrylate can be stored for several months at 5° after stabilization with 0.1% hydroquinone or, alternatively, II can be readily prepared from the stable methyl 2,3-dichloropropionate by dehydrohalogenation with quinoline⁶.

Treatment of methyl 2-chloroacrylate with labeled potassium cyanide in aqueous methanol in the presence of sodium bicarbonate yielded a mixture of

*More correctly, the label is statistically distributed at both C-1 and C-4 positions. A significant amount of the doubly labeled species is therefore formed at high specific activities.

Scheme 1



methyl trans-3-cyanoacrylate (IIIa) and methyl cis-3-cyanoacrylate (IIIb) in the approximate ratio of 5:1. The reported yield of the esters IIIa and IIIb totals 40%⁷ for a four hour reaction time. However, extension of the reaction time to 16 hours and utilization of the acids IVa and IVb** raised the conversion of potassium cyanide to 70-80%. The free acids IVa and IVb probably resulted from the hydrolysis of the corresponding esters under the mildly basic conditions.

The reaction mixture was diluted with water, acidified and extracted with ethyl acetate. Evaporation of the extract afforded a crude mixture of IIIa, IIIb, IVa, IVb and starting material II. For the purpose of conversion to fumaric acid, the mixture was not fractionated prior to hydrolysis with 6N

** The postulated presence of both trans and cis isomers of 3-cyanoacrylic acid is based on the observation by GC of both trans and cis methyl esters upon methylation with methyl iodide in methanol in the presence of potassium carbonate. The possibility exists that isomerization may have occurred under these conditions. Reaction with diazomethane yielded only less volatile adducts.

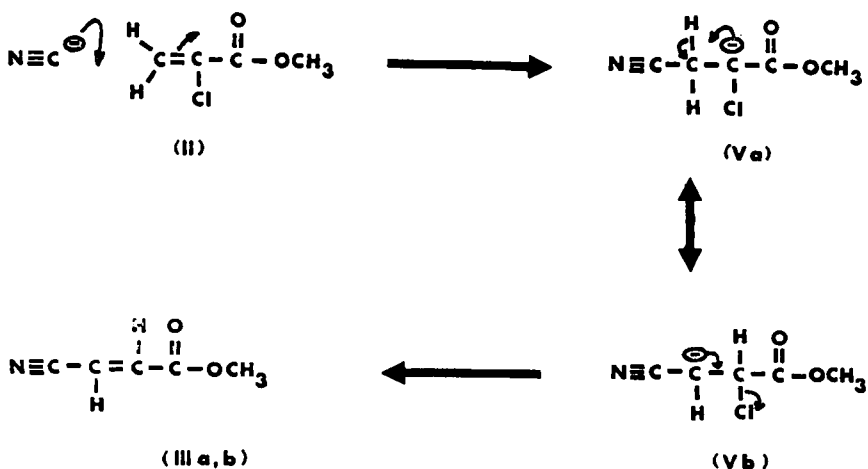
hydrochloric acid. Concentration of the hydrolysate gave crystalline fumaric acid in 45% yield based on potassium cyanide. The mother liquor contained an additional quantity of fumaric acid-1- ^{14}C (11%) and 9% D,L-malic acid-1- ^{14}C and 4- ^{14}C . The latter is the product of hydration of fumaric acid and becomes more dominant on prolonged refluxing with 6 N hydrochloric acid.

The fumaric acid was free of maleic acid or malic acid by paper chromatography. The radiochemical purity exceeded 99%. Dehydration with phosphorus pentoxide by the method of Nystrom et al.¹ produced maleic anhydride in 95% yield.

PROPOSED MECHANISM

The mechanism of this reaction appears to be unknown. It is postulated that a Michael addition of cyanide occurs first, since activated unsaturated esters undergo facile base-catalyzed 1,4-addition of hydrogen cyanide⁸. Equilibration of the resultant carbanion Va to the more stable carbanion Vb is followed by β -elimination of chloride ion with regeneration of the double bond. (Scheme 1)

S c h e m e 2



However, the expected addition product could not be detected by gas chromatography in the reaction mixture under standard conditions or at lower pH values of 4, 5, 6 and 7 under conditions where elimination of chloride would be less favorable. No reaction occurred at pH 4 and increasing amounts of IIIa and IIIb were formed at higher pH values.

EXPERIMENTAL

Melting points were determined in Mel-Temp apparatus and are corrected. Boiling points are uncorrected. Infrared spectra were obtained in KBr pellets in a Perkin-Elmer 137 spectrophotometer. Gas chromatographic analyses were run in a Perkin-Elmer F-21 gas chromatograph under the following conditions: detector, FID; carrier gas, N₂ at 0.60 Kg/cm²; injection temperature, 175°; column temperature, 112°; manifold temperature; 126°. Column: 1/4" O.D. X 6' aluminum. Column packing: SE-30 (19%) on 80-100 mesh Chromosorb B (acid washed and treated with dimethyl chlorosilane). Thin-layer chromatograms were run on cellulose layers containing a fluorescent indicator. Solvent system, 95% ethanol-water-concentrated ammonia (80:10:20). Whatman 3MM paper was used for ascending chromatography in the same solvent system. Spots were visualized under U.V. (254nm) and by spraying with bromocresol green (0.04% in ethanol).

Methyl 2-chloroacrylate (II). This compound was prepared from methyl 2,3-dichloropropionate (Eastman Kodak Corporation) according to the procedure of Marvel *et al.* (6). The methyl 2-chloroacrylate, b.p. 71-73°/120mm, was distilled from the reaction mixture in 81% yield and was used without further purification. GC analysis indicated a purity of 98-99% (R_T=1.7 min. and impurities at R_T 0.7 and 2.2 min.). Stabilization with 0.1% hydroquinone permitted storage at 5° for several months.

Acknowledgement

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Methyl-trans and cis-3-cyanoacrylates (IIIa and IIIb): The published procedure⁷ was used, except for an increase in the reaction time from four to sixteen hours⁹. The reaction mixture was diluted with water and extracted three times with ethyl acetate. Fractionation of the extract afforded pure methyl trans-3-cyanoacrylate (IIIa), b.p. 85-88°/31mm, m.p. 31-33°, GC: $R_T=2.0$ min. (lit. (9) m.p. 32-34°) and a mixed fraction, b.p. 65-84°/10mm, containing 74% IIIa and 26% IIIb, $R_T=4.0$ min. (the published retention times for IIIa and IIIb on a Carbowax column at 140° and 25 psi are 2.9 and 9.3 min., respectively).

Cis and trans 3-cyanoacrylic acid (IVa and IVb): The mixed acids were isolated by extraction of the acidified aqueous layer after the extraction of the methyl esters. The extracts were washed three times with a saturated NaCl solution, dried (Na_2SO_4) and evaporated to dryness. The residue (Ig) was chromatographed on 20g silica gel EM (article #7734) in chloroform. The chloroform and chloroform-methanol (95:5) eluates were evaporated and the residue crystallized from benzene-petroleum ether. The crystalline 3-cyanoacrylic acids weighed 290 mg (6% yield) m.p. 100-101.5°; neutralization equivalent, (calc'd 97.1), 98.2; I.R. (cm^{-1}) 3100 broad, 2235 (C≡N) 1722 (C=O) 1628 (C=C). Lit.¹⁰ m.p. 98.0°; N.E. found: 98.4, Silica gel TLC (Quantum 21F; chloroform-methanol-formic acid, 95:5:1) Rf 0.3-0.4.

Methylation of IVa and IVb: a. Via methyl iodide/ K_2CO_3 in methanol for 16 hours¹⁰: GC indicated two components corresponding to IIIa and IIIb. b. Via diazomethane in dichloromethane: the reaction product was a less volatile crystalline solid which was not identified and probably resulted from 1,3-dipolar addition of diazomethane to III or IV.¹²

Fumaric Acid-1-¹⁴C: The reaction of K^{14}CN with II was conducted in a 50cc flask sealed with a rubber septum. Methyl 2-chloroacrylate (1.070g, 8.85mM), sodium bicarbonate (0.748g, 8.85mM), methanol (1.6 cc), water (0.7cc) and 0.02 cc aniline were added to the flask. The septum was attached and securely fastened, since slight pressure developed in the course of the reaction. The

K¹⁴CN, 166 mg unlabeled and 410 mg labeled KCN, specific activity 16.8 mC/mM totaling 106 mC in 8.85 mM was dissolved in 1.4 cc water and added dropwise via a syringe to the stirred reaction mixture while cooling in an ice-water bath. The mixture was stirred at 0-5° for one hour and at ambient temperature for 16 hours. After dilution with water (10cc) and acidification with 6N HCl (3.5cc), the mixture was purged briefly with N₂ to remove H¹⁴CN which was trapped in 1N NaOH (11.4mC). Extraction with ethyl acetate (4x5cc) yielded an extract containing 91 mC. The spent aqueous layer contained 4.5 mC. The ethyl acetate extracts were concentrated in vacuo (aspirator) at 25-30° to a light brown oil containing II, IIIa, IIIb, IVa and IVb. The mixture was hydrolyzed without prior fractionation.

The crude concentrate was refluxed in 6N HCl (21cc) for two hours. The hydrolysate was evaporated to dryness in vacuo (aspirator) and the residue was crystallized from water to yield 502 mg fumaric acid-1-¹⁴C as tan colored crystals. The chemical and radiochemical yields were 45% based on the KCN introduced (specific activity 10.8 mC/mM; 48 mC). TLC and paper chromatography indicated a radiochemical purity of greater than 99% and no detectable amounts of labeled maleic acid or D,L-malic acid. The filtrate and washes (26.3 mC) were chromatographed on Whatman 3MM paper and yielded an additional 12 mC of fumaric acid (11%) and 9 mC D,L-malic acid (8.5%). Conversion of the fumaric acid-1-¹⁴C to maleic anhydride-1-¹⁴C by the method of Nystrom et al.¹ proceeded in 95% yield.

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